

WHAT IS CLAIMED IS:

1. A method for detecting the presence of a target nucleic acid in a sample comprising:
  - 5 providing a sample comprising a target nucleic acid and non-target nucleic acids;
  - providing a first complementary nucleic acid which is complementary to a first nucleotide sequence in a target nucleic acid;
  - 10 providing a second complementary nucleic acid which is complementary to a second nucleotide sequence in said target nucleic acid;
  - 15 providing a first sequestering agent which specifically interacts with said first complementary nucleic acid, wherein said first sequestering agent reduces the likelihood that said first complementary nucleic acid will hybridize to said non-target nucleic acids;
  - hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid;
  - 20 hybridizing said second complementary nucleic acid to said second nucleotide sequence in said target nucleic acid;
  - ligating said first complementary nucleic acid which is hybridized to said first nucleotide sequence in said target nucleic acid to said second complementary nucleic acid which is hybridized to said second nucleotide sequence in said target nucleic acid, thereby forming a ligation product; and
  - 25 detecting the presence of said ligation product.
2. The method of Claim 1, wherein said first complementary nucleic acid and said second complementary nucleic acid are present on the same nucleic acid molecule such that said ligation product comprises a circular nucleic acid.
3. The method of Claim 1, wherein a first oligonucleotide comprises said first complementary nucleic acid and a second distinct oligonucleotide comprises said second complementary nucleic acid.
4. The method of Claim 1, wherein said first nucleotide sequence in said target nucleic acid and said second nucleotide sequence in said target nucleic acid are immediately adjacent to one another on said target nucleic acid.

5. The method of Claim 1, wherein said first nucleotide sequence in said target nucleic acid and said second nucleotide sequence in said target nucleic acid are not immediately adjacent to one another on said target nucleic acid.

6. The method of Claim 1, further comprising providing a second sequestering agent which interacts with said second complementary nucleic acid, thereby reducing the likelihood that said second oligonucleotide will hybridize to said non-target nucleic acids.

7. The method of Claim 1, wherein said first sequestering agent comprises a nucleic acid comprising a nucleotide sequence which is complementary to at least a portion of said first complementary nucleic acid, wherein a duplex between said first complementary nucleic acid and said sequestering agent has a melting point intermediate to the melting point of a duplex between said first complementary nucleic acid and said non-target nucleic acids and the melting point of a duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid.

8. The method of Claim 7, wherein the residue at the 3' terminus of said first sequestering agent lacks a 3-hydroxyl group such that the 5' phosphate of said first complementary nucleic acid cannot be ligated to the 3' terminus of said sequestering agent.

9. The method of Claim 8, wherein the residue at the 3' terminus of said first sequestering agent comprises a dideoxysugar.

10. The method of Claim 8, wherein the residue at the 3' terminus of said sequestering agent comprises a blocking group such that the 5' phosphate of said first complementary nucleic acid cannot be ligated to the 3' terminus of said first sequestering agent.

11. The method of Claim 10, wherein said blocking group is selected from the group consisting of NH<sub>2</sub>, F, Cl, Br, NO<sub>2</sub>, OR<sub>1</sub>, O-C(O)-R<sub>2</sub>, NHR<sub>3</sub>, alkyl, and H where R<sub>1</sub> is selected from the group consisting of carbonyl, alkyl, acyl, alkene, or heteroalkyl, said alkyl, acyl, alkene or heteroalkyl group being substituted or unsubstituted, R<sub>2</sub> is selected from the group consisting of alkyl, acyl, alkene, or heteroalkyl, said alkyl, acyl, alkene or heteroalkyl group being substituted or unsubstituted and R<sub>3</sub> is selected from the group consisting of alkyl, acyl, alkene, or heteroalkyl, said alkyl, acyl, alkene or heteroalkyl group being substituted or unsubstituted.

12. The method of Claim 8, wherein said sequestering agent comprises a stem loop.

13. The method of Claim 12, wherein the 3' terminus is in the duplex portion of said stem loop.

14. The method of Claim 7, wherein said sequestering agent has a linear conformation.

5 15. The method of Claim 7, wherein said sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said sequestering agent.

16. The method of Claim 7, wherein said sequestering agent lacks a 5' phosphate.

10 17. The method of Claim 16, wherein the nucleotide at the 5' terminus of said sequestering agent comprises a blocking group which prevents said nucleotide from being ligated to another nucleic acid, wherein said blocking group is selected from the group consisting of NH<sub>2</sub>, F, Cl, Br, NO<sub>2</sub>, OR<sub>1</sub>, O-C(O)-R<sub>2</sub>, NHR<sub>3</sub>, alkyl, and H where R<sub>1</sub> is selected from the group consisting of carbonyl, alkyl, acyl, alkene, or heteroalkyl, said 15 alkyl, acyl, alkene or heteroalkyl group being substituted or unsubstituted, R<sub>2</sub> is selected from the group consisting of alkyl, acyl, alkene, or heteroalkyl, said alkyl, acyl, alkene or heteroalkyl group being substituted or unsubstituted and R<sub>3</sub> is selected from the group consisting of alkyl, acyl, alkene, or heteroalkyl, said alkyl, acyl, alkene or heteroalkyl group being substituted or unsubstituted.

20 18. The method of Claim 7, wherein said sequestering agent reduces the accessibility of the 3' hydroxyl of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said sequestering agent.

25 19. The method of Claim 6, wherein said first equestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said first sequestering agent and wherein said second sequestering agent reduces the accessibility of the 3' hydroxyl of said second complementary nucleic acid to a ligase when said second complementary nucleic acid is hybridized to said second sequestering agent.

30 20. The method of Claim 1, wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at

least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid.

10        21.      The method of Claim 6 wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and wherein said hybridizing step comprises hybridizing said second complementary nucleic acid to said second nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid.

complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid.

5        22. The method of Claim 7, wherein said target nucleic acid comprises a single nucleotide polymorphism.

10      23. The method of Claim 22, wherein said first complementary nucleic acid comprises the variable nucleotide of said single nucleotide polymorphism such that said first complementary nucleic acid is fully complementary to a first allele of said single nucleotide polymorphism.

24. The method of Claim 23, wherein said variable nucleotide position is at the 3' terminus of said first complementary nucleic acid.

15      25. The method of Claim 23, further comprising:

providing a third complementary nucleic acid wherein said third complementary nucleic acid comprises the variable nucleotide of said single nucleotide polymorphism such that said third complementary nucleic acid is fully complementary to a second allele of said single nucleotide polymorphism,

20      providing a second sequestering agent comprising a nucleic acid which is complementary to at least a portion of said third complementary nucleic acid and wherein said at least a portion of said third complementary nucleic acid includes said variable nucleotide of said second allele of said single nucleotide polymorphism;

25      wherein said at least a portion of said first complementary nucleic acid to which said first sequestering agent is complementary includes said variable nucleotide of said first allele of said single nucleotide polymorphism.

26. The method of Claim 1, wherein said sequestering agent is present in excess of said first complementary nucleic acid.

27. The method of Claim 26, wherein said sequestering agent is present in a molar excess of about 1.5 to about 2 relative to said first complementary nucleic acid.

30      28. The method of Claim 1, wherein the concentrations of said first complementary nucleic acid and said sequestering agent are selected so that at equilibrium about 5% or less of said first nucleotide sequence in said target nucleic acid is unoccupied by said first complementary nucleic acid.

29. The method of Claim 1, wherein said sample comprises genomic DNA.
30. The method of Claim 1, wherein said step of detecting the presence of said ligation product comprises performing a PCR reaction to amplify said ligation product, thereby generating an amplified ligation product and detecting said amplified ligation product.  
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31. The method of Claim 1, wherein said step of detecting the presence of said ligation product comprises circularizing a nucleic acid template, thereby generating a circular molecule if said ligation product is present and detecting said circular molecule.
32. The method of Claim 1, wherein said step of detecting the presence of said ligation product comprises performing a rolling circle amplification reaction, thereby generating an amplification product and detecting said amplification product.  
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33. The method of Claim 1, wherein said step of detecting the presence of said ligation product comprises detecting hybridization of said ligation product or of a nucleic acid indicative of the presence of said ligation product to a solid support.
- 15
34. The method of Claim 33, wherein said nucleic acid indicative of the presence of said ligation product comprises an identifier tag wherein said identifier tag is generated only when said target nucleotide sequence corresponding to said identifier tag is present in said sample.
- 20
35. The method of Claim 33, wherein said solid support comprises a universal detector.
36. The method of Claim 35, wherein said universal detector comprises detection probes coupled to a detecting component which measures hybridization of said ligation product or of said nucleic acid indicative of the presence of said ligation product to any of said detection probes.  
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37. The method of Claim 36, wherein said detecting component is electrochemical, fluorescent, colorimetric, radiometric or magnetic.
38. The method of Claim 37, wherein said detecting component is electrochemical.
39. The method of Claim 36, wherein said detection probes coupled to said detecting component are attached to a surface, film, or particle.  
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40. The method of Claim 39, wherein said detection probes are attached to said surface, film, or particle by covalent bonds, ionic bonds, electrostatic interactions or adsorptive interactions.

41. The method of Claim 39, wherein said detection probes are attached to a particle such as a bead.

42. The method of Claim 39, wherein said detection probes are attached to a plurality of particles.

5 43. The method of Claim 35, wherein said universal detector comprises an array of detection probes coupled to detecting components, said array comprising electrodes attached to a substrate.

44. The method of Claim 43, wherein said electrodes are gold or carbon.

45. The method of Claim 44, wherein said electrodes are gold.

10 46. The method of Claim 43, further comprising measuring hybridization to any said detection probe by ruthenium amperometry.

47. The method of Claim 43, wherein said electrodes are coated with protein which can be bound by oligonucleotides derivatized with a moiety that binds said protein that coats said electrode.

15 48. The method of Claim 47, wherein said electrodes are coated with avidin such that said electrodes can be bound by biotin-labeled oligonucleotides.

49. An isolated nucleic acid comprising:

a first nucleotide sequence complementary to a probe nucleic acid such that said probe nucleic acid can hybridize to said nucleic acid, wherein at least a portion of said probe nucleic acid is complementary to a target nucleic acid;

20 a second nucleotide sequence comprising a region which forms a structure such that one terminus of said nucleic acid is in proximity to one terminus of said probe nucleic acid when said probe nucleic acid is hybridized to said nucleic acid and wherein a duplex between said probe nucleic acid and said first nucleotide sequence has a melting point intermediate to the melting point of a duplex between said probe nucleic acid and said target nucleic acid and the melting point of a duplex between said probe nucleic acid and non-target nucleic acids.

25 50. The nucleic acid of Claim 49 wherein said nucleic acid reduces the accessibility of the 5' phosphate of said probe nucleic acid to a ligase when said probe nucleic acid is hybridized to said nucleic acid.

30 51. The nucleic acid of Claim 49, wherein said nucleic acid lacks a 5' phosphate.

52. The nucleic acid of 51, wherein the nucleotide at the 5' terminus of said nucleic acid comprises a blocking group which prevents said nucleotide from being ligated to another nucleic acid, wherein said blocking group is selected from the group consisting of NH<sub>2</sub>, F, Cl, Br, NO<sub>2</sub>, OR<sub>1</sub>, O-C(O)-R<sub>2</sub>, NHR<sub>3</sub>, alkyl, and H where R<sub>1</sub> is selected from the group consisting of carbonyl, alkyl, acyl, alkene, or heteroalkyl, said alkyl, acyl, alkene or heteroalkyl group being substituted or unsubstituted, R<sub>2</sub> is selected from the group consisting of alkyl, acyl, alkene, or heteroalkyl, said alkyl, acyl, alkene or heteroalkyl group being substituted or unsubstituted and R<sub>3</sub> is selected from the group consisting of alkyl, acyl, alkene, or heteroalkyl, said alkyl, acyl, alkene or heteroalkyl group being substituted or unsubstituted.

10 53. The nucleic acid of Claim 49, wherein the terminus of said nucleic acid which is adjacent to said probe nucleic acid when said probe nucleic acid is hybridized to said nucleic acid contains a moiety thereon which cannot be ligated to said probe nucleic acid by a ligase.

15 54. The nucleic acid of Claim 53, wherein said moiety which cannot be ligated to said probe nucleic acid by a ligase is on the residue at the 3' terminus of said nucleic acid.

20 55. The nucleic acid of Claim 54, wherein the 3' terminus of said nucleic acid lacks a 3-hydroxyl group such that the 5' phosphate of said probe nucleic acid cannot be ligated to the 3' terminus of said nucleic acid.

56. The nucleic acid of Claim 55, wherein the residue at the 3' terminus of said nucleic acid comprises a dideoxysugar.

25 57. The nucleic acid of Claim 55, wherein the residue at the 3' terminus of said nucleic acid comprises a blocking group such that the 5' phosphate of said probe nucleic acid cannot be ligated to the 3' terminus of said nucleic acid.

58. The nucleic acid of Claim 57, wherein said blocking group is selected from the group consisting of NH<sub>2</sub>, F, Cl, Br, NO<sub>2</sub>, OR<sub>1</sub>, O-C(O)-R<sub>2</sub>, NHR<sub>3</sub>, alkyl, and H where R<sub>1</sub> is selected from the group consisting of carbonyl, alkyl, acyl, alkene, or heteroalkyl, said alkyl, acyl, alkene or heteroalkyl group being substituted or unsubstituted, R<sub>2</sub> is selected from the group consisting of alkyl, acyl, alkene, or heteroalkyl, said alkyl, acyl, alkene or heteroalkyl group being substituted or unsubstituted and R<sub>3</sub> is selected from the group consisting of alkyl, acyl, alkene, or heteroalkyl, said alkyl, acyl, alkene or heteroalkyl group being substituted or unsubstituted.

59. The nucleic acid of Claim 49, wherein said structure comprises a stem loop.
60. The nucleic acid of Claim 59, wherein the 3' terminus of said nucleic acid is in the duplex portion of said stem loop.
61. The nucleic acid of Claim 49, wherein said first nucleotide sequence which  
5 is complementary to at least a portion of said probe nucleic acid comprises a sequence which is polymorphic in the genome of an organism containing said target nucleic acid.
62. The nucleic acid of Claim 61, wherein said first nucleotide sequence which  
is complementary to at least a portion of said probe nucleic acid comprises the variable  
nucleotide of a single nucleotide polymorphism in the genome of an organism containing  
10 said target nucleic acid.
63. The nucleic acid of Claim 62, wherein said variable nucleotide is  
complementary to the 3' terminus of said probe nucleic acid.
64. A method for generating a ligation product comprising:
  - providing a first nucleic acid and a second nucleic acid;
  - 15 forming sequestration complexes, said sequestration complexes comprising either said first nucleic acid or said second nucleic acid specifically complexed with a sequestering agent or comprising both said first nucleic acid and said second nucleic acid specifically complexed with sequestering agents;
  - hybridizing said first nucleic acid and said second nucleic acid to a third  
20 nucleic acid containing nucleotide sequences complementary to said first and second nucleic acids, wherein the stability of a duplex between said third nucleic acid and said first nucleic acid or said second nucleic acid is greater than the stability of said sequestration complexes; and
  - 25 ligating said first nucleic acid to said second nucleic acid while said first nucleic acid and said second nucleic acid are hybridized to said third nucleic acid.
65. A method for determining whether a sample contains a target nucleic acid comprising:
  - providing a sample to be tested for the presence of a target nucleic acid, said sample containing non-target nucleic acids;
  - 30 providing a first complementary nucleic acid which is complementary to a first nucleotide sequence in a target nucleic acid;
  - providing a second complementary nucleic acid which is complementary to a second nucleotide sequence in said target nucleic acid;

providing a first sequestering agent which specifically interacts with said first complementary nucleic acid, wherein said first sequestering agent reduces the likelihood that said first complementary nucleic acid will hybridize to said non-target nucleic acids;

5 performing a hybridization reaction such that said first complementary nucleic acid will hybridize to said first nucleotide sequence in said target nucleic acid if said target nucleic acid is present in said sample;

10 performing a hybridization reaction such that said second complementary nucleic acid will hybridize to said second nucleotide sequence in said target nucleic acid if said target nucleic acid is present in said sample;

providing a ligase such that if said first complementary nucleic acid is hybridized to said first nucleotide sequence in said target nucleic acid and said second complementary nucleic acid is hybridized to said second nucleotide sequence in said target nucleic acid, a ligation product will be generated; and

15 determining whether said ligation product has been generated.

66. A method for determining whether a target nucleic acid is present in a sample comprising:

providing a sample to be tested for the presence of a target nucleic acid said sample comprising non-target nucleic acids;

20 performing an amplification reaction on said sample, thereby generating an amplified target nucleic acid if said sample contains said target nucleic acid;

25 providing a probe comprising a first end comprising a first complementary nucleic acid complementary to a first nucleotide sequence in said target nucleic acid, a second end comprising a second complementary nucleic acid complementary to a second nucleotide sequence in said target nucleic acid, a promoter recognized by an RNA polymerase, and a nucleotide sequence complementary to a tag sequence indicative of the presence of said target nucleic acid in said sample;

30 providing a first sequestering agent which specifically interacts with said first complementary nucleic acid, wherein said first sequestering agent reduces the likelihood that said first complementary nucleic acid will hybridize to said non-target nucleic acids;

contacting said sample with said probe, such that said first complementary nucleic acid will hybridize to said first nucleotide sequence in said amplified target

nucleic acid and said second complementary nucleic acid will hybridize to said second nucleotide sequence in said amplified target nucleic acid and said probe will adopt a circular conformation if said target nucleic acid is present in said sample;

5                   ligating the ends of said circular probe together if said circular probe is present;

performing a transcription reaction with said RNA polymerase, thereby generating a transcript comprising said tag if said sample contains said target nucleic acid; and

determining whether said transcript has been generated.

10         67. The method of Claim 66, wherein said RNA polymerase is selected from the group consisting of T7 RNA polymerase, R4 RNA polymerase, T3 RNA polymerase, *E. coli* RNA polymerase, SP6 RNA polymerase, RNA polymerase II and RNA polymerase III.

15         68. The method of Claim 66, wherein said promoter is positioned 3' of said first complementary nucleic acid and said nucleotide sequence complementary to said tag is positioned 5' of said second complementary nucleic acid.

69. The method of Claim 66, further comprising performing an exonuclease digestion after ligating the ends of said probe together.

70. The method of Claim 66, further comprising providing a second sequestering agent which specifically interacts with said second complementary nucleic acid, wherein said second sequestering agent reduces the likelihood that said second complementary nucleic acid will hybridize to said non-target nucleic acids.

71. The method of Claim 66, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said first sequestering agent.

25         72. The method of Claim 66, wherein said first sequestering agent reduces the accessibility of the 3' hydroxyl of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said sequestering agent.

73. The method of Claim 70, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when 30         said first complementary nucleic acid is hybridized to said first sequestering agent and said second sequestering agent reduces the accessibility of the 3' hydroxyl of said second complementary nucleic acid to a ligase when said second complementary nucleic acid is hybridized to said second sequestering agent.

74. The method of Claim 66, wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid.

15 75. The method of Claim 70 wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and wherein said hybridizing step comprises hybridizing said second complementary nucleic acid to said second nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the

melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid.

10        76.      A method for determining whether a target nucleic acid is present in a sample comprising:

              providing a sample to be tested for the presence of a target nucleic acid said sample comprising non-target nucleic acids;

15        performing an amplification reaction on said sample, thereby generating an amplified target nucleic acid if said sample contains said target nucleic acid;

20        providing a probe comprising a first end comprising a first complementary nucleic acid complementary to a first nucleotide sequence in said target nucleic acid, a second end comprising a second complementary nucleic acid complementary to a second nucleotide sequence in said target nucleic acid, a promoter recognized by an RNA polymerase, and a nucleotide sequence complementary to a tag sequence indicative of the presence of said target nucleic acid in said sample;

25        providing a first sequestering agent which specifically interacts with said first complementary nucleic acid, wherein said first sequestering agent reduces the likelihood that said first complementary nucleic acid will hybridize to said non-target nucleic acids;

30        contacting said sample with said probe, such that said first complementary nucleic acid will hybridize to said first nucleotide sequence in said amplified target nucleic acid and said second complementary nucleic acid will hybridize to said second nucleotide sequence in said amplified target nucleic acid and said probe will adopt a circular conformation if said target nucleic acid is present in said sample;

              ligating the ends of said circular probe together if said circular probe is present;

              linearizing said circularized probe;

performing a transcription reaction with said RNA polymerase, thereby generating a transcript comprising said tag if said sample contains said target nucleic acid; and

determining whether said transcript has been generated

5 77. The method of Claim 76, wherein said RNA polymerase is selected from the group consisting of T7 RNA polymerase, R4 RNA polymerase, T3 RNA polymerase, *E. coli* RNA polymerase, SP6 RNA polymerase, RNA polymerase II and RNA polymerase III.

78. The method of Claim 76, wherein said promoter is positioned 3' of said first complementary nucleic acid and said nucleotide sequence complementary to said tag is  
10 positioned 5' of said second complementary nucleic acid.

79. The method of Claim 76, further comprising performing an exonuclease digestion after ligating the ends of said probe together.

80. The method of Claim 76, further comprising providing a second sequestering agent which specifically interacts with said second complementary nucleic acid, wherein said second sequestering agent reduces the likelihood that said second complementary nucleic acid will hybridize to said non-target nucleic acids.  
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81. The method of Claim 76, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said first sequestering agent.

20 82. The method of Claim 76, wherein said first sequestering agent reduces the accessibility of the 3' hydroxyl of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said sequestering agent.

83. The method of Claim 80, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when  
25 said first complementary nucleic acid is hybridized to said first sequestering agent and said second sequestering agent reduces the accessibility of the 3' hydroxyl of said second complementary nucleic acid to a ligase when said second complementary nucleic acid is hybridized to said second sequestering agent.

84. The method of Claim 76, wherein said hybridizing step comprises  
30 hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a

temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid.

10        85.      The method of Claim 80 wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and wherein said hybridizing step comprises hybridizing said second complementary nucleic acid to said second nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid and a temperature at least 30 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid.

between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid.

5        86. A method for determining whether a target nucleic acid is present in a sample comprising:

providing a sample to be tested for the presence of a target nucleic acid said sample comprising non-target nucleic acids;

10      performing an amplification reaction on said sample, thereby generating an amplified target nucleic acid if said sample contains said target nucleic acid;

15      providing a probe comprising a first end comprising a first complementary nucleic acid complementary to a first nucleotide sequence in said target nucleic acid, a second end comprising a second complementary nucleic acid complementary to a second nucleotide sequence in said target nucleic acid, and a nucleotide sequence comprising a sequence complementary to or identical to a tag sequence indicative of the presence of said target nucleic acid in said sample;

20      providing a first sequestering agent which specifically interacts with said first complementary nucleic acid, wherein said first sequestering agent reduces the likelihood that said first complementary nucleic acid will hybridize to said non-target nucleic acids;

25      contacting said sample with said probe, such that said first complementary nucleic acid will hybridize to said first nucleotide sequence in said amplified target nucleic acid and said second complementary nucleic acid will hybridize to said second nucleotide sequence in said amplified target nucleic acid and said probe will adopt a circular conformation if said target nucleic acid is present in said sample;

30      ligating the ends of said circular probe together if said circular probe is present;

linearizing said circularized probe; and

determining whether said linearized probe has been generated.

35      87. The method of Claim 86, wherein said nucleotide sequence comprising said tag is positioned 5' of said second complementary nucleic acid.

36      88. The method of Claim 86, further comprising performing an exonuclease digestion after ligating the ends of said probe together.

89. The method of Claim 86, further comprising providing a second sequestering agent which specifically interacts with said second complementary nucleic acid, wherein said second sequestering agent reduces the likelihood that said second complementary nucleic acid will hybridize to said non-target nucleic acids.

5 90. The method of Claim 86, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said first sequestering agent.

10 91. The method of Claim 86, wherein said first sequestering agent reduces the accessibility of the 3' hydroxyl of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said sequestering agent.

15 92. The method of Claim 89, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said first sequestering agent and said second sequestering agent reduces the accessibility of the 3' hydroxyl of said second complementary nucleic acid to a ligase when said second complementary nucleic acid is hybridized to said second sequestering agent.

20 93. The method of Claim 86, wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature 25 of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary 30 nucleic acid and said first nucleotide sequence in said target nucleic acid.

94. The method of Claim 89 wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at

least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and wherein said hybridizing step comprises hybridizing said second complementary nucleic acid to said second nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid.

95. A method for determining whether a target nucleic acid is present in a sample comprising:

providing a sample to be tested for the presence of a target nucleic acid said sample comprising non-target nucleic acids;

30 providing a probe comprising a first end comprising a first complementary nucleic acid complementary to a first nucleotide sequence in said target nucleic acid, a second end comprising a second complementary nucleic acid complementary to a second nucleotide sequence in said target nucleic acid, a promoter recognized

by an RNA polymerase, and a nucleotide sequence complementary to a tag sequence indicative of the presence of said target nucleic acid in said sample;

5 providing a first sequestering agent which specifically interacts with said first complementary nucleic acid, wherein said first sequestering agent reduces the likelihood that said first complementary nucleic acid will hybridize to said non-target nucleic acids;

10 contacting said sample with said probe, such that said first complementary nucleic acid will hybridize to said first nucleotide sequence in said sample and said second complementary nucleic acid will hybridize to said second nucleotide sequence in said sample and said probe will adopt a circular conformation if said target nucleic acid is present in said sample;

15 ligating the ends of said circular probe together if said circular probe is present;

amplifying at least a portion of said probe comprising said promoter and said 15 nucleotide sequence complementary to said tag;

performing a transcription reaction with said RNA polymerase, thereby generating a transcript comprising said tag if said sample contains said target nucleic acid; and

determining whether said transcript has been generated

20 96. The method of Claim 95, wherein said RNA polymerase is selected from the group consisting of T7 RNA polymerase, R4 RNA polymerase, T3 RNA polymerase, *E. coli* RNA polymerase, SP6 RNA polymerase, RNA polymerase II and RNA polymerase III.

97. The method of Claim 95, wherein said promoter is positioned 3' of said first complementary nucleic acid and said nucleotide sequence complementary to said tag is positioned 5' of said second complementary nucleic acid.

25 98. The method of Claim 95, further comprising performing an exonuclease digestion after ligating the ends of said probe together.

99. The method of Claim 95, further comprising providing a second sequestering agent which specifically interacts with said second complementary nucleic acid, wherein said second sequestering agent reduces the likelihood that said second complementary nucleic acid will hybridize to said non-target nucleic acids.

100. The method of Claim 95, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said first sequestering agent.

101. The method of Claim 95, wherein said first sequestering agent reduces the 5 accessibility of the 3' hydroxyl of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said sequestering agent.

102. The method of Claim 99, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said first sequestering agent and said second sequestering agent reduces the accessibility of the 3' hydroxyl of said second complementary nucleic acid to a ligase when said second complementary nucleic acid is hybridized to said second sequestering agent.

103. The method of Claim 95, wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid.

104. The method of Claim 99 wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said

target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said  
5 first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and wherein said hybridizing step comprises hybridizing said second complementary nucleic acid to said second nucleotide sequence in said target nucleic acid at a temperature selected from the  
10 group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 15  
15 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting  
20 temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid.

105. A method for determining whether a target nucleic acid is present in a sample comprising:

25 providing a sample to be tested for the presence of a target nucleic acid said sample comprising non-target nucleic acids;

providing a probe comprising a first end comprising a first complementary nucleic acid complementary to a first nucleotide sequence in said target nucleic acid, a second end comprising a second complementary nucleic acid complementary to a second nucleotide sequence in said target nucleic acid, and a nucleotide sequence comprising a sequence complementary to or identical to a tag sequence indicative of the presence of said target nucleic acid;  
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providing a first sequestering agent which specifically interacts with said first complementary nucleic acid, wherein said first sequestering agent reduces the

likelihood that said first complementary nucleic acid will hybridize to said non-target nucleic acids;

5                   contacting said sample with said probe, such that said first complementary nucleic acid will hybridize to said first nucleotide sequence in said sample and said second complementary nucleic acid will hybridize to said second nucleotide sequence in said sample and said probe will adopt a circular conformation if said target nucleic acid is present in said sample;

10                  ligating the ends of said circular probe together if said circular probe is present;

amplifying at least a portion of said probe comprising said tag, thereby generating an amplification product if said sample contains said target nucleic acid; and

determining whether said amplification product has been generated.

106. The method of Claim 105, wherein said nucleotide sequence comprising 15 said tag is positioned 5' of said second complementary nucleic acid.

107. The method of Claim 105, further comprising performing an exonuclease digestion after ligating the ends of said probe together.

108. The method of Claim 105, further comprising providing a second sequestering agent which specifically interacts with said second complementary nucleic acid, wherein said second sequestering agent reduces the likelihood that said second complementary nucleic acid will hybridize to said non-target nucleic acids.

109. The method of Claim 105, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said first sequestering agent.

25                  110. The method of Claim 105, wherein said first sequestering agent reduces the accessibility of the 3' hydroxyl of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said sequestering agent.

111. The method of Claim 108, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when 30 said first complementary nucleic acid is hybridized to said first sequestering agent and said second sequestering agent reduces the accessibility of the 3' hydroxyl of said second complementary nucleic acid to a ligase when said second complementary nucleic acid is hybridized to said second sequestering agent.

112. The method of Claim 105, wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said first  
5 complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide  
10 sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid.

113. The method of Claim 108 wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the  
20 melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and wherein said hybridizing step comprises hybridizing said second complementary nucleic acid to said  
25 second nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the  
30

melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid.

10        114. A method for determining whether a target nucleic acid is present in a sample comprising:

              providing a sample to be tested for the presence of a target nucleic acid said sample comprising non-target nucleic acids;

15        performing an amplification reaction on said sample, thereby generating an amplified target nucleic acid if said sample contains said target nucleic acid;

              providing a first complementary nucleic acid comprising a promoter recognized by an RNA polymerase positioned 3' of a sequence complementary to a first nucleotide sequence in said target nucleic acid ;

20        providing a second complementary nucleic acid comprising a sequence complementary to a tag sequence indicative of the presence of said target nucleic acid in said sample positioned 5' of a sequence complementary to a second nucleotide sequence in said target nucleic acid, wherein said second complementary nucleic acid is on a separate molecule from said first complementary nucleic acid;

25        providing a first sequestering agent which specifically interacts with said first complementary nucleic acid, wherein said first sequestering agent reduces the likelihood that said first complementary nucleic acid will hybridize to said non-target nucleic acids;

30        contacting said sample with said first complementary nucleic acid and said second complementary nucleic acid, such that said first complementary nucleic acid will hybridize to said first nucleotide sequence in said amplified target nucleic acid and said second complementary nucleic acid will hybridize to said second nucleotide sequence in said amplified target nucleic acid;

ligating said first complementary nucleic acid to said second complementary nucleic acid if said target nucleic acid is present in said sample;

5 performing a transcription reaction with said RNA polymerase, thereby generating a transcript comprising said tag if said sample contains said target nucleic acid; and

determining whether said transcript has been generated.

115. The method of Claim 114, wherein said RNA polymerase is selected from the group consisting of T7 RNA polymerase, R4 RNA polymerase, T3 RNA polymerase, *E. coli* RNA polymerase, SP6 RNA polymerase, RNA polymerase II and RNA polymerase III.

10 116. The method of Claim 114, wherein said first complementary nucleic acid is resistant to digestion by a 3' exonuclease and said second complementary nucleic acid is resistant to digestion by a 5' exonuclease and wherein said method further comprises performing a digestion with a 3' exonuclease and a 5' exonuclease after ligating said first complementary nucleic acid to said second complementary nucleic acid if said sample 15 contains said target nucleic acid.

117. The method of Claim 114, further comprising providing a second sequestering agent which specifically interacts with said second complementary nucleic acid, wherein said second sequestering agent reduces the likelihood that said second complementary nucleic acid will hybridize to said non-target nucleic acids.

20 118. The method of Claim 114, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said first sequestering agent.

25 119. The method of Claim 117, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said first sequestering agent and said second sequestering agent reduces the accessibility of the 3' hydroxyl of said second complementary nucleic acid to a ligase when said second complementary nucleic acid is hybridized to said second sequestering agent.

120. The method of Claim 114, wherein said hybridizing step comprises 30 hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a

temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid.

10        121. The method of Claim 117 wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and wherein said hybridizing step comprises hybridizing said second complementary nucleic acid to said second nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid and a temperature at least 30 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid.

between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid.

5        122. A method for determining whether a target nucleic acid is present in a sample comprising:

providing a sample to be tested for the presence of a target nucleic acid said sample comprising non-target nucleic acids;

10        performing an amplification reaction on said sample, thereby generating an amplified target nucleic acid if said sample contains said target nucleic acid;

providing a first complementary nucleic acid comprising a sequence complementary to a first nucleotide sequence in said target nucleic acid ;

15        providing a second complementary nucleic acid comprising a tag sequence indicative of the presence of said target nucleic acid in said sample positioned 5' of a sequence complementary to a second nucleotide sequence in said target nucleic acid, wherein said second complementary nucleic acid is on a separate molecule from said first complementary nucleic acid;

20        providing a first sequestering agent which specifically interacts with said first complementary nucleic acid, wherein said first sequestering agent reduces the likelihood that said first complementary nucleic acid will hybridize to said non-target nucleic acids;

25        contacting said sample with said first complementary nucleic acid and said second complementary nucleic acid, such that said first complementary nucleic acid will hybridize to said first nucleotide sequence in said amplified target nucleic acid and said second complementary nucleic acid will hybridize to said second nucleotide sequence in said amplified target nucleic acid

ligating said first complementary nucleic acid to said second complementary nucleic acid if said target nucleic acid is present in said sample, thereby generating a ligation product if said target nucleic acid is present in said sample; and

30        determining whether said ligation product has been generated.

123. The method of Claim 122, wherein said first complementary nucleic acid is resistant to digestion by a 3' exonuclease and said second complementary nucleic acid is resistant to digestion by a 5' exonuclease and wherein said method further comprises

performing a digestion with a 3' exonuclease and a 5' exonuclease after ligating said first complementary nucleic acid to said second complementary nucleic acid if said sample contains said target nucleic acid.

124. The method of Claim 122, further comprising providing a second 5 sequestering agent which specifically interacts with said second complementary nucleic acid, wherein said second sequestering agent reduces the likelihood that said second complementary nucleic acid will hybridize to said non-target nucleic acids.

125. The method of Claim 122, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when 10 said first complementary nucleic acid is hybridized to said first sequestering agent.

126. The method of Claim 124, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said first sequestering agent and said second sequestering agent reduces the accessibility of the 3' hydroxyl of said second 15 complementary nucleic acid to a ligase when said second complementary nucleic acid is hybridized to said second sequestering agent.

127. The method of Claim 122, wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at 20 least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature 25 of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary 30 nucleic acid and said first nucleotide sequence in said target nucleic acid.

128. The method of Claim 124 wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at

least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees

5 Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and wherein said hybridizing step comprises hybridizing said second complementary nucleic acid to said second nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature

10 of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second

15 nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second

20 nucleotide sequence in said target nucleic acid.

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129. A method for determining whether a target nucleic acid is present in a sample comprising:

providing a sample to be tested for the presence of a target nucleic acid said sample comprising non-target nucleic acids;

30 providing a first complementary nucleic acid comprising a promoter recognized by an RNA polymerase positioned 3' of a sequence complementary to a first nucleotide sequence in said target nucleic acid;

providing a second complementary nucleic acid comprising a sequence complementary to a tag sequence indicative of the presence of said target nucleic acid in said sample positioned 5' of a sequence complementary to a second nucleotide sequence in said target nucleic acid, wherein said second complementary nucleic acid is on a separate molecule from said first complementary nucleic acid;

5 providing a first sequestering agent which specifically interacts with said first complementary nucleic acid, wherein said first sequestering agent reduces the likelihood that said first complementary nucleic acid will hybridize to said non-target nucleic acids;

10 contacting said sample with said first complementary nucleic acid and said second complementary nucleic acid, such that said first complementary nucleic acid will hybridize to said first nucleotide sequence in said target nucleic acid and said second complementary nucleic acid will hybridize to said second nucleotide sequence in said target nucleic acid

15 ligating said first complementary nucleic acid to said second complementary nucleic acid if said target nucleic acid is present in said sample, thereby generating a ligation product if said target nucleic acid is present in said sample;

amplifying said ligation product if present;

20 performing a transcription reaction on said amplified ligation product with said RNA polymerase, thereby generating a transcript comprising said tag if said sample contains said target nucleic acid; and

determining whether said transcript has been generated.

130. The method of Claim 129, wherein said RNA polymerase is selected from the group consisting of T7 RNA polymerase, R4 RNA polymerase, T3 RNA polymerase, *E. coli* RNA polymerase, SP6 RNA polymerase, RNA polymerase II and RNA polymerase III.

25 131. The method of Claim 129, wherein said first complementary nucleic acid is resistant to digestion by a 3' exonuclease and said second complementary nucleic acid is resistant to digestion by a 5' exonuclease and wherein said method further comprises performing a digestion with a 3' exonuclease and a 5' exonuclease after ligating said first complementary nucleic acid to said second complementary nucleic acid if said sample contains said target nucleic acids.

30 132. The method of Claim 129, further comprising providing a second sequestering agent which specifically interacts with said second complementary nucleic

acid, wherein said second sequestering agent reduces the likelihood that said second complementary nucleic acid will hybridize to said non-target nucleic acids.

133. The method of Claim 129, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when  
5 said first complementary nucleic acid is hybridized to said first sequestering agent.

134. The method of Claim 132, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when  
said first complementary nucleic acid is hybridized to said first sequestering agent and said  
second sequestering agent reduces the accessibility of the 3' hydroxyl of said second  
10 complementary nucleic acid to a ligase when said second complementary nucleic acid is  
hybridized to said second sequestering agent.

135. The method of Claim 129, wherein said hybridizing step comprises  
hybridizing said first complementary nucleic acid to said first nucleotide sequence in said  
target nucleic acid at a temperature selected from the group consisting of a temperature at  
15 least 5 degrees Celsius below the melting temperature of the duplex between said first  
complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a  
temperature at least 10 degrees Celsius below the melting temperature of the duplex  
between said first complementary nucleic acid and said first nucleotide sequence in said  
target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature  
20 of the duplex between said first complementary nucleic acid and said first nucleotide  
sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the  
melting temperature of the duplex between said first complementary nucleic acid and said  
first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees  
Celsius below the melting temperature of the duplex between said first complementary  
25 nucleic acid and said first nucleotide sequence in said target nucleic acid.

136. The method of Claim 132 wherein said hybridizing step comprises  
hybridizing said first complementary nucleic acid to said first nucleotide sequence in said  
target nucleic acid at a temperature selected from the group consisting of a temperature at  
least 5 degrees Celsius below the melting temperature of the duplex between said first  
30 complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a  
temperature at least 10 degrees Celsius below the melting temperature of the duplex  
between said first complementary nucleic acid and said first nucleotide sequence in said  
target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature

of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees  
5 Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and wherein said hybridizing step comprises hybridizing said second complementary nucleic acid to said second nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature  
10 of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second  
15 nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second  
20 nucleotide sequence in said target nucleic acid.

137. A method for determining whether a target nucleic acid is present in a sample comprising:

providing a sample to be tested for the presence of a target nucleic acid said sample comprising non-target nucleic acids;

25 providing a first complementary nucleic acid comprising a sequence complementary to a first nucleotide sequence in said target nucleic acid ;

providing a second complementary nucleic acid comprising a sequence complementary to or identical to a tag indicative of the presence of said target nucleic acid in said sample positioned 5' of a sequence complementary to a second nucleotide sequence in said target nucleic acid, wherein said second complementary nucleic acid is on a separate molecule from said first complementary nucleic acid;  
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providing a first sequestering agent which specifically interacts with said first complementary nucleic acid, wherein said first sequestering agent reduces the

likelihood that said first complementary nucleic acid will hybridize to said non-target nucleic acids;

5 contacting said sample with said first complementary nucleic acid and said second complementary nucleic acid, such that said first complementary nucleic acid will hybridize to said first nucleotide sequence in said target nucleic acid and said second complementary nucleic acid will hybridize to said said second nucleotide sequence in said target nucleic acid

10 ligating said first complementary nucleic acid to said second complementary nucleic acid if said target nucleic acid is present in said sample, thereby generating a ligation product if said target nucleic acid is present in said sample;

amplifying said ligation product if present; and

determining whether said ligation product has been generated.

138. The method of Claim 137, wherein said first complementary nucleic acid is resistant to digestion by a 3' exonuclease and said second complementary nucleic acid is resistant to digestion by a 5' exonuclease and wherein said method further comprises performing a digestion with a 3' exonuclease and a 5' exonuclease after ligating said first complementary nucleic acid to said second complementary nucleic acid if said sample contains said target nucleic acid.

139. The method of Claim 137, further comprising providing a second sequestering agent which specifically interacts with said second complementary nucleic acid, wherein said second sequestering agent reduces the likelihood that said second complementary nucleic acid will hybridize to said non-target nucleic acids.

140. The method of Claim 137, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when 25 said first complementary nucleic acid is hybridized to said first sequestering agent.

141. The method of Claim 139, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said first sequestering agent and said second sequestering agent reduces the accessibility of the 3' hydroxyl of said second complementary nucleic acid to a ligase when said second complementary nucleic acid is hybridized to said second sequestering agent.

142. The method of Claim 137, wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said

target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid.

143. The method of Claim 139 wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and wherein said hybridizing step comprises hybridizing said second complementary nucleic acid to said second nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 15

degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid.

144. A method for determining whether a target nucleic acid is present in a sample comprising:

- 10 providing a sample to be tested for the presence of a target nucleic acid said sample comprising non-target nucleic acids;
- 15 providing a probe comprising a first end comprising a first complementary nucleic acid complementary to a first nucleotide sequence in said target nucleic acid and a second end comprising a second complementary nucleic acid complementary to a second nucleotide sequence in said target nucleic acid;
- 20 providing a first sequestering agent which specifically interacts with said first complementary nucleic acid, wherein said first sequestering agent reduces the likelihood that said first complementary nucleic acid will hybridize to said non-target nucleic acids;
- 25 contacting said sample with said probe, such that said first complementary nucleic acid will hybridize to said first nucleotide sequence in said target nucleic acid and said second complementary nucleic acid will hybridize to said second nucleotide sequence in said target nucleic acid and said probe will adopt a circular conformation if said target nucleic acid is present in said sample;
- 30 ligating the ends of said circular probe together if said circular probe is present;
- 35 performing a rolling circle amplification procedure using said circularized and ligated probe as a template, thereby generating an amplification product if said sample contains said target nucleic acid; and
- 40 determining whether said amplification product has been generated.

145. The method of Claim 144, wherein said probe further comprises a sequence complementary to a tag indicative of the presence of said target nucleic acid, and said

determining step comprises determining whether an amplification product comprising said tag has been generated.

146. The method of Claim 144, further comprising performing an exonuclease digestion after ligating the ends of said probe together.

5 147. The method of Claim 144, further comprising providing a second sequestering agent which specifically interacts with said second complementary nucleic acid, wherein said second sequestering agent reduces the likelihood that said second complementary nucleic acid will hybridize to said non-target nucleic acids.

10 148. The method of Claim 144, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said first sequestering agent.

149. The method of Claim 144, wherein said first sequestering agent reduces the accessibility of the 3' hydroxyl of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said sequestering agent.

15 150. The method of Claim 147, wherein said first sequestering agent reduces the accessibility of the 5' phosphate of said first complementary nucleic acid to a ligase when said first complementary nucleic acid is hybridized to said first sequestering agent and said second sequestering agent reduces the accessibility of the 3' hydroxyl of said second complementary nucleic acid to a ligase when said second complementary nucleic acid is 20 hybridized to said second sequestering agent.

151. The method of Claim 144, wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees

Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid.

152. The method of Claim 147 wherein said hybridizing step comprises hybridizing said first complementary nucleic acid to said first nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said first complementary nucleic acid and said first nucleotide sequence in said target nucleic acid and wherein said hybridizing step comprises hybridizing said second complementary nucleic acid to said second nucleotide sequence in said target nucleic acid at a temperature selected from the group consisting of a temperature at least 5 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 10 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 15 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid, a temperature at least 20 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid and a temperature at least 25 degrees Celsius below the melting temperature of the duplex between said second complementary nucleic acid and said second nucleotide sequence in said target nucleic acid.